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13. ABSTRACT ***(Maximum 200 Words)***

The c-erbB2 (or HER-2, *neu*) gene encodes a 185-kDa transmembrane glycoprotein (p185), which belongs to the EGF-r family . The c-erbB2 gene was found to be amplified and/or overexpressed in approximately 30% of human breast carcinomas. Our previous studies demonstrated that c-erbB2 overexpression can enhance metastatic potential and confer increased chemoresistance to breast cancer cells, therby leading to poor clinical outcome in breast cancer patients. Our recent studies demonstrated that overexpression of the c-erbB2 gene can protect human breast cancer cells from apoptosis induced by the chemotherapeutic agent Taxol. One of the mechanisms is overexpression of c-erbB2 can upregulate $p21^{Cip1}$, which inhibits taxol-mediated $p34^{Cdc2}$ activation, delays cell entrance to G2/M phase, and thereby inhibits taxol-induced apoptosis. This new finding provided an explanation on c-erbB2 mediated chemoresistance of breast cancer cells and also explored the importance of $p21^{Cip1}$ in G2/M phase transition. Since we only use the 435 and its c-erbB2 transfectants in this study, we would like to further confirm this in other c-erbB2 overexpression breast cancer cell lines with different genetic background. Then we will find out whether other $p34^{Cdc2}$ regulators contribute to taxol resistance.

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FOREWORD

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Tong Tong 6/12/2000

PI - Signature Date

Table of Contents

Progression Reports for 1999-2000

A.Introduction

The c-erbB2 (or HER-2, *neu*) gene encodes a 185-kDa transmembrane glycoprotein (p185), which belongs to the EGF-r family . The c-erbB2 gene was found to be amplified and/or overexpressed in approximately 30% of human breast carcinomas. Our previous studies demonstrated that c-erbB2 overexpression can enhance metastatic potential and confer increased chemoresistance to breast cancer cells, therby leading to poor clinical outcome in breast cancer patients. Our recent studies demonstrated that overexpression of the c-erbB2 gene can protect human breast cancer cells from apoptosis induced by the chemotherapeutic agent Taxol. One of the mechanisms is overexpression of c-erbB2 can upregulate $p21^{Cip1}$, which inhibits taxol-mediated $p34^{Cdc2}$ activation, delays cell entrance to G2/M phase, and thereby inhibits taxol-induced apoptosis. This new finding provided an explanation on c-erbB2 mediated chemoresistance of breast cancer cells and also explored the importance of $p21^{Cip1}$ in G2/M phase transition. Since we only use the 435 and its c-erbB2 transfectants in this study, we would like to further confirm this in other c-erbB2 overexpression breast cancer cell lines with different genetic background. Then we will find out whether other $p34^{Cdc2}$ regulators contribute to taxol resistance.

B. Specific Aims

The specific Aims 1 and 2 have been modified. Since we found that the activity of $p34^{Cdc2}$ is important of taxol-induced apoptosis. In our early finding that $p21^{Cip1}$

play an important role in 435.eB transfectants to inhibit the activity of $p34^{Cdc2}$. We plan to study other c-erbB2 overexpression breast cancer cell lines to see whether this is a general phenomenal.

Aim 1. Identify $p34^{Cdc2}$ regulators involved in Taxol resistance in breast cancer cell lines.

Aim 2. Investigate these $p34^{Cdc2}$ regulators are direct involved in Taxol resistance in breast cancer cell lines.

C. Study Results and Significance

Aim 1. Identify $p34^{Cdc2}$ regulators involved in Taxol resistance in breast cancer cell lines.

To determine whether the activity of $p34^{Cdc2}$ is important in other breast cancer cell lines, we use two taxol sensitive breast cancer cell lines MDA-MB-435, MDA-MB-231 and two taxol resistant cell lines BT 474, MDA-MB-361 (Appendices Fig.1), to compare their $p34^{Cdc2}$ activity. Since phosphorylation of tyrosine 15 residue is an indication of inhibition of $p34^{Cdc2}$ activity, I used immunoblotting with phospho-tyr-15 specific antibody to determine the activity of $p34^{Cdc2}$ in these four cell lines (Appendices Fig.2). The phosphorylation of tyrosine 15 is higher in taxol resistant cell lines compared with taxol sensitive cell lines. Since Wee1 and Cdc25C are two important regulators to regulate the phosphorylation status on $p34^{Cdc2}$ tyrosine 15 residue, I screened these two factors by using immunoblotting. Interestingly ,Wee1 expression level is higher in BT474 and 361 cell lines (Fig.3.). I also screened other $p34^{Cdc2}$ regulators $p21^{Cip1}$ and cyclin B1(1). As expected, $p21^{Cip1}$ expression level is higher in BT 474 and 361 cell lines (Fig.4.) whereas

cyclin B1 expression level is much lower in these two cell lines(1). From above data, multiple regulators are involved in regulating the taxol sensitivity. Currently, aim 1 already been finished, I will start the aim 2 study.

D. Plans

Aim 1. Identify $p34^{Cdc2}$ regulators involved in Taxol resistance in breast cancer cell lines.

Finished.

Aim 2. Investigate these $p34^{Cdc2}$ regulators are direct involved in Taxol resistance in breast cancer cell lines.

I will design or construct anti-sense p21 oligonucleotide, anti-sense wee1 plasmid and cyclin B1 plasmid, transfect each one of them into the taxol resistant cell lines BT474 and 361. Then to investigate whether bring down or bring up one of these factors can resensitize the breast cancer cell to taxol induced apoptosis.

E. Conclusions

With the data we already had, overexpression of c-erbB2 can confer the taxol induced apoptosis by upregulation of $p21^{Cip1}$, which in turn inhibits the taxol mediated activation of $p34^{Cdc2}$. But in other breast cancer cell lines, other $p34^{Cdc2}$ regulators like Wee1 and cyclin B1 also play a role in $p34^{Cdc2}$ inhibition. These leading us to find out that $p34^{Cdc2}$ may be an important target for taxol resistance.

F. Reference

1. Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T. J., and Hung, M.-C. Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of $p21^{Cip1}$, which inhibits $p34^{Cdc2}$ kinase, Molecular Cell. *2:* 581-591, 1998a.

Appendices

FIGURE 1. DNA ladder assay of breast cancer cell lines sensitivity to taxol induced apoptosis

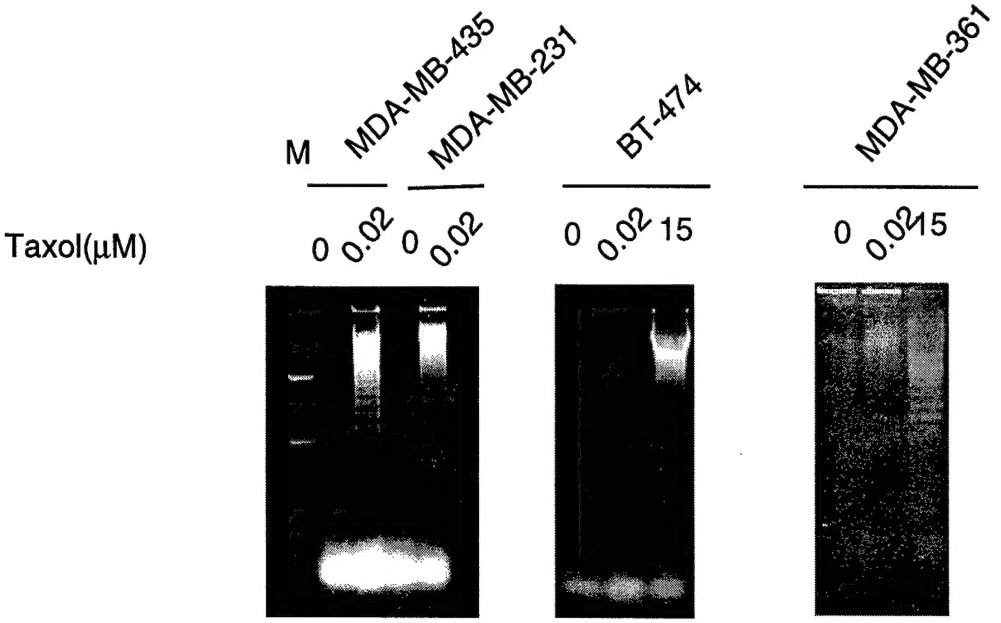

FIGURE 2. Western Blotting of phosphorylation of Cdc2 in different breast cancer cell lines

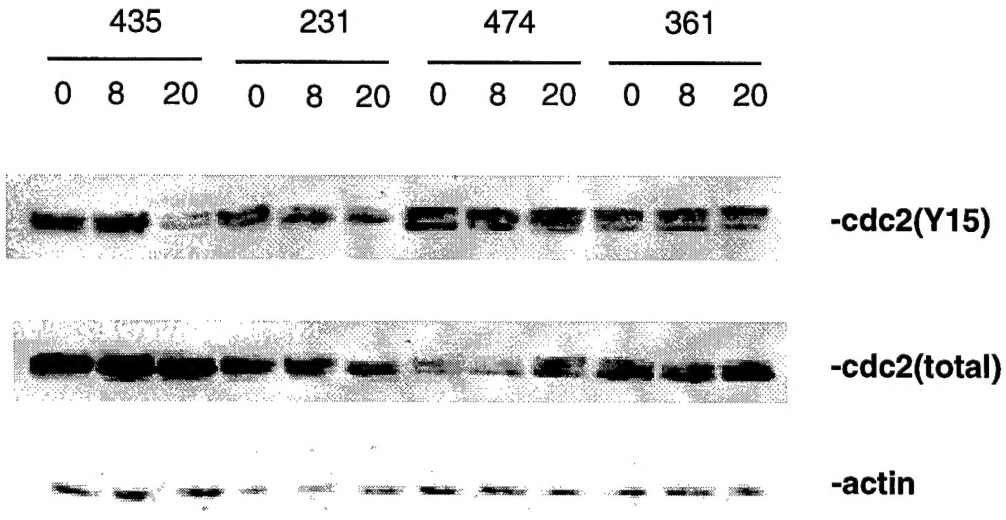

FIGURE 3. Western blotting of Wee1 and Cdc25C expression level in breast cancer cell lines

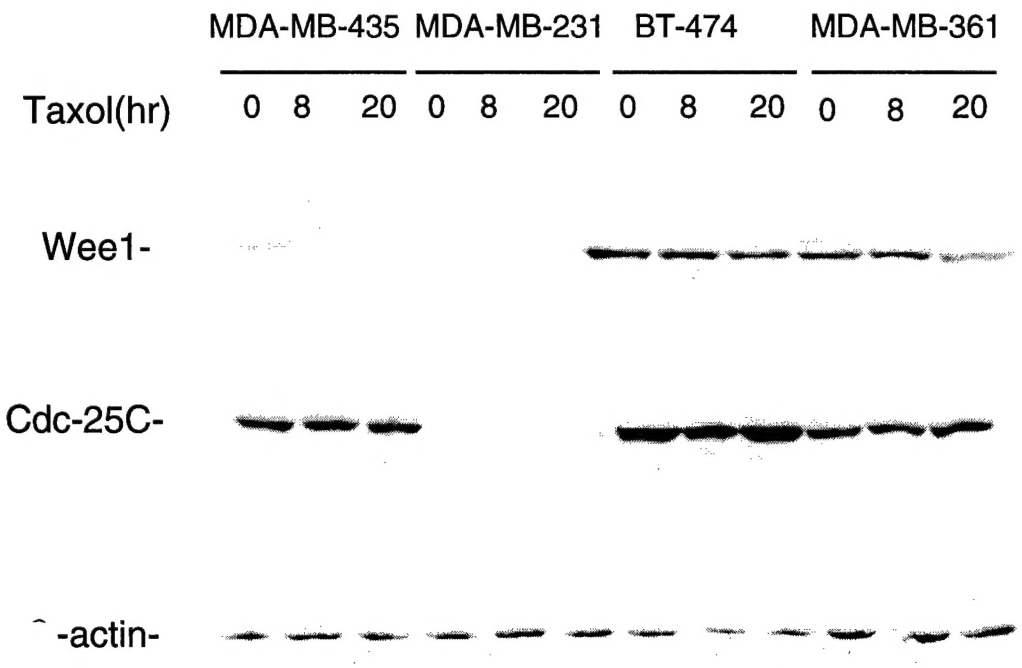

FIGURE 4. Western blotting of p21 expression level in breast cancer cell lines